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A Critical Review of the Factors Available for the Identification and Determination of Mānuka Honey

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Abstract
Methods for the determination of the authenticity of samples of mānuka honey are reviewed. Suggestions are made as to how to authenticate, or otherwise, the label claims for a given sample of mānuka honey.

Keywords Mānuka honey · Authenticity · Chemical markers · Analytical methods

Introduction

Honey Definitions and Compositional Standards

Honey, a sweet, natural product produced by honeybees (*Apis mellifera*) is defined as 'the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature' (Codex Alimentarius 1981; EU Council 2002). Bees process the collected material with enzymes, including diastase (amylases) and invertase (α-glucosidase), dehydrate and deposit it in honeycombs to ripen and mature. Thus, honey is a concentrated aqueous solution of 'invert' sugar (the monosaccharides glucose and fructose) and has been used by mankind since ancient times, with more than 95% of its dry mass comprising of sugars and water (Manyi-Loh et al. 2011). Honey also typically contains a wide range of saccharides, amino acids, proteins, organic acids, vitamins, minerals, enzymes, polyphenols and pollen. Some of these components are due to the maturation of the honey, the addition of components from the bees or are derived from the plants (Anklam 1997). However, the botanical source and the geographical area from where the honey originated are connected to the variation of honey's composition, along with the species of the bee, season in which the honey is produced and its means of storage (Jandrić et al. 2017).

Key compositional criteria for honey are set out in a Codex standard including maxima for moisture, sucrose and water-insoluble solids and minima for the sum of fructose and glucose (Codex Alimentarius 1981). The EU Directive for honey (EU Council 2002) includes these and several more, for electrical conductivity, maxima for free acidity and hydroxymethylfurfural and minima for diastase activity. The Directive differentiates between honey (blossom or nectar honey in Codex) and honeydew honey, the latter mainly from excretions of plant sucking insects (*Hemiptera*) or secretions of living parts of plants. Further descriptive terms permitted for various honeys include filtered honey, comb honey, chunk honey or cut comb-in honey and baker's honey. Except in the case of filtered honey and baker's honey, the product names may be supplemented by information referring to floral or vegetable origin (if the product comes wholly or mainly from the indicated source and possesses the organoleptic, physicochemical and microscopic (mainly the pollen present) characteristics of the source); regional, territorial or topographical origin (if the product comes entirely from the indicated source); or with specific quality criteria. The country or countries of origin where the honey have been harvested must be indicated on the label although where appropriate the supplementary information 'blend of EU honeys', 'blend of non-EU honeys' or 'blend of EU and non-EU honeys' may be given instead.

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Honey Adulteration

There are many means of adulterating honey, including the addition of cheap sugars and syrups after collection of honey from hives (Daniele et al. 2012), overfeeding bees with saccharides or invert saccharide derivatives, to increase honey production (Kolayli et al. 2012) and the falsification of the floral or geographical origin of the honey (Daniele et al. 2012). Honey mislabelling and fraud is a global issue (Moore et al. 2012; Fairchild et al. 2003). In the face of media reports that more mānuka honey is sold worldwide than is produced in New Zealand (e.g. Leake 2017), the New Zealand Ministry for Primary Industries, NZ-MPI, started in 2013 research to develop a set of high level characteristics for *mānuka-type* honey (MPI 2013) but considered, on the then available data, that it did not appear possible to give robust, widely accepted and scientifically validated parameters for *monofloral* mānuka honey (MPI 2014). They then initiated a scientific programme aimed to establish appropriate characteristics and robust, usable and independently validated testing for mānuka honey and reported in 2017 (MPI 2017a, b, c).

Alongside the above, focused more widely than on mānuka honey, the European Commission organised in 2015 a coordinated control plan to assess the market prevalence of honey adulterated with sugars and honeys mislabelled with regard to their botanical source or geographical origin (EU 2015). The 28 Member States, Switzerland and Norway collected over 2000 samples of honey and reported 19% of samples showed non-compliance and 13% were classified as 'suspicion of non-compliance'. These latter were considered to return unusual or questionable results. The Commission noted current methods have limitations with regard to exogenous sugars used to adulterate honey. Hence, the Commission's Joint Research Centre & Institute for Reference Materials and Measurements, JRC, was mandated to further analyse samples that were not characterised as adulterated with the current tests.

The JRC report was released in April 2017 (Aries et al. 2016). Of the 2264 honey samples collected in the control plan, 45% were from retailers and the reminder represented all stages of the supply chain. Member States, MS, checked compliance with sensory characteristics and pollen profiles (tier 1). Those that were found compliant in tier 1 were submitted to sugar analysis (tier 2), and those that were found still compliant in tier 2 were subjected to stable carbon isotope analysis by elemental analysis-isotope ratio mass spectrometry (δ^{13}C EA-IRMS) and a combination of EA-IRMS and liquid chromatography coupled to isotope ratio mass spectrometry (δ^{13}C EA/LC-IRMS), for the detection of added sugars (tier 3). When LC-IRMS was not available to MS, JRC provided this initial analysis.

After the completion of the above control plan, JRC was sent samples that had been found compliant by the tests carried out in the MS amounting to 893 samples (40% of all collected samples) for analysis by EA/LC-IRMS. The JRC findings were that 14% (127/893) of the submitted honey samples did not conform to published benchmark purity criteria (δ^{13}C ratios of individual sugars, protein and the percent of peak areas of oligosaccharides indicating that foreign sugars may have been added (Elflein and Raezke 2008). Interestingly, the JRC findings indicated that the most important purity criterion to detect adulteration with rice syrup was the oligosaccharide peak area.

Hence, the above exercise indicated a total of some 38% of the honey samples examined in the EU were non-compliant with authenticity criteria. Although this high percentage may have been inflated by the targeted nature of the surveillance, it nevertheless represents a worrying figure that lends credence to media reports questioning the authenticity of significant amounts of mānuka honey on the global market.

Mānuka Honey

Mānuka honey is the monofloral product of *Leptospermum scoparium*, a New Zealand native plant, said to possess 'non-peroxide anti-bacterial activity' (Mavric et al. 2008), making it greatly sought-after world-wide. Around 10,000–12,000 t of honey is produced each year in New Zealand, in which 6000–8000 t of which is available for export is mainly mānuka honey (Rogers et al. 2014).

In addition to the antimicrobial activity in honey which is credited to hydrogen peroxide, the product of catalytic activity of glucose oxidases on water (Fearnley et al. 2012), mānuka honey possesses additional antibacterial activity, due to a phytochemical derived from the nectar of the flower (Wallace et al. 2010). Dihydroxyacetone, present in the nectar of mānuka flowers, is the direct precursor for methylglyoxal in mānuka honey, which is the major bactericide that contributes to mānuka's additional bioactive properties (Adams et al. 2008, 2009). Mānuka honey is sought after because of its elevated antibacterial activity, which is classified in trade using a 'unique mānuka factor' (UMF). UMF corresponds with the concentration (*w/v* %) of an aqueous phenol solution exhibiting the same antibacterial activity in a well diffusion assay against *Staphylococcus aureus* (Wallace et al. 2010).

The Current New Zealand Position: MPI Chosen Mānuka Markers and Their Analytical Methods

The long awaited statement by the New Zealand authorities (MPI 2017a, b, c) now gives the number and amounts of chemicals and the complex methods for their determination that they consider to be appropriate to allow a sample of honey to be described as authentic mānuka honey. The scientific

basis to the New Zealand criteria are promised and awaited with interest. Thus, it is appropriate to raise the possibility of the use of fewer, but more specific markers and the use of simpler, structure sensitive, analytical methods for producers, importers and food trade analysts.

The MPI science programme found that a combination of five attributes (four chemical compounds, and one DNA marker from mānuka pollen) is required to separate mānuka honey from other honey types and to identify monofloral and multifloral mānuka honey. The four chemicals are 3-phenyllactic acid, 2′-methoxyacetophenone, 2-methoxybenzoic acid and 4-hydroxyphenyllacetic acid, determined by LC-MS/MS (MPI 2017b). The DNA is determined by multiplex qPCR (MPI 2017c).

The Criteria for Monofloral Mānuka Honey

The test for monofloral mānuka honey requires all of the five attributes. If the honey fails to meet 1 or more of the attributes, it is not monofloral mānuka honey—but may still pass the test for multifloral mānuka honey.

The following compounds all need to be present and at the levels stated:

- 3-Phenyllactic acid at a level greater or equal to 400 mg kg^{-1}
- 2′-Methoxyacetophenone at a level greater than or equal to 5 mg kg^{-1}
- 2-Methoxybenzoic acid at a level greater than or equal to 1 mg kg^{-1}
- 4-Hydoxyphenyllactic acid at a level greater or equal to 1 mg kg^{-1}

In DNA from mānuka pollen, the level required is less than Cq 36, which is approximately 3 fg μL^{-1}.

The Criteria for Multifloral Mānuka Honey

The test for multifloral mānuka honey requires all of the five attributes. If the honey fails to meet one or more of the attributes, it is not mānuka honey.

The following chemicals need to be present and at the levels stated:

- 3-Phenyllactic acid at level greater than or equal to 20 mg kg^{-1} but less than 400 mg kg^{-1}
- 2′-Methoxyacetophenone at a level greater than or equal to 5 mg kg^{-1}
- 2-Methoxyphenyllactic acid at a level greater than or equal to 1 mg kg^{-1}
- 4-Hydroxyphenyllactic acid at a level greater than or equal to 1 mg kg^{-1}

In DNA from mānuka pollen, the level required is less than Cq 36, which is approximately 3 fg μL^{-1}.

These criteria can be usefully expressed in tabular format, see Table 1, when it is seen that the sole distinction between monofloral and multifloral mānuka is in their 3-phenyllactic acid contents.

Although 3-phenyllactic acid is not confined solely to mānuka plants, MPI found that its concentration range was sufficiently characteristic to differentiate mānuka honey from other New Zealand honey types and monofloral from multifloral mānuka honey. In December 2017, in response to industry feedback NZ-MPI increased the required concentration of 2′-methoxyacetophenone from 1 to 5 mg kg^{-1}. Since this compound has only been found in mānuka plants to date, this move is aimed to make it more difficult for anyone to attempt blending different types of honey with mānuka honey to meet the definition. NZ-MPI also introduced requirements in December 2017 to improve how bee products are traced through the New Zealand supply chain and ensure New Zealand bee products comply with importing country requirements (MPI 2017d).

A problem for food importers and port of entry authorities is the cost and time for data acquisition that applying the MPI 5 compound criteria for genuine mānuka honey entails as they require the services of both advanced chemical analysis and molecular biology laboratories. A further weakness in the approach, for the prevention of fraud, is that the distinction between mono- or multifloral mānuka depends on the amount present of a readily synthesised compound. For these reasons, it is considered important to consider the use difficult to synthesise mānuka marker compounds, to avoid fraud from their addition, coupled with cheaper and more rapid methods of analysis.

Potentially Quantitative Markers for Mānuka Honey Authenticity

Methylglyoxal and Dihydroxyacetone

The occurrence and formation of 1,2-dicarbonyl compounds in honey as a result of sugar degradation were first discussed

Table 1 The five compounds and specified limits to characterise mono- and multifloral mānuka honey by MPI

Compounds	Monofloral	Multifloral
3-Phenyllactic acid	≥ 400 mg kg^{-1}	≥ 20, < 400 mg kg^{-1}
2′-Methoxyacetophenone	≥ 5 mg kg^{-1}	≥ 5 mg kg^{-1}
2-Methoxybenzoic acid	≥ 1 mg kg^{-1}	≥ 1 mg kg^{-1}
4-Hydroxyphenyllactic acid	≥ 1 mg kg^{-1}	≥ 1 mg kg^{-1}
DNA	< Cq 36 3 fg μL^{-1}	< Cq 36 3 fg μL^{-1}

by Weigel et al. (2004). The methylglyoxal (MGO) content is low in honey apart from mānuka honey where it can be at a concentration of up to 760 mg kg^{-1} (Mavric et al. 2008; Atrott et al. 2012). The MGO within mānuka honey is not due to sugar degradation but from the presence of dihydroxyacetone (DHA) which exists in varying amounts in mānuka flower nectar (Adams et al. 2009) and converts to MGO during honey maturation (Spiteri et al. 2017). Since DHA has been used as a food additive and in cosmetic self-tanning creams, it is commercially available in bulk which weakens the case for both it and MGO as reliable chemical markers for mānuka honey.

Methyl Syringate

Mānuka honey contains a unique phenolic compound, methyl syringate (MS), which is said to display the scavenging activity of super-oxides and inhibitory effects on aflatoxin production. As MS has been found in some honeys from Sardinia and is widely distributed in plants, it is not suitable for use as a sole marker for mānuka honey (Kato et al. 2014a, b).

Leptosperin (Also Known as Leptosin)

Leptosperin, a novel glycoside of methyl syringate, was discovered to be specifically in mānuka honey in 2012 (Kato et al. 2012). It is relatively heat stable making it a dependable chemical marker (Kato et al. 2014a, b; Bong et al. 2017). It may be determined by immune-chromatographic assay (Kato et al. 2014a, b, 2016) or by fluorescence (Bong et al. 2017). The compound can be made available for use as an internal standard but the synthesis is complex (Aitken et al. 2013), thus rendering it less likely to be deployed as a honey adulterant.

DNA

Methods have been described to detect specific floral pollen DNA fragments in honey using PCR and metabarcoding (Jain et al. 2013; Lalhmangaihi et al. 2014; Guertler et al. 2014; Bruni et al. 2015; Soares et al. 2015; Hawkins et al. 2015; Torricelli et al. 2016; Prosser and Hebert 2017) and recently advocated as a marker for mānuka honey (MPI 2017c). These markers indicate the presence but not the amount of mānuka honey in a sample. The problem arises because the amount of pollen or DNA per gramme of each floral honey component collected by the bees is an unknown and likely to vary with the variety of *Leptospermum scoparium* flowers, their maturity and the weather.

Laboratory-Based Methods for the Quantification of Selected Mānuka Marker Compounds

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) can provide quantitative data and molecular structural information on key components with little sample preparation for honey samples (Consonni and Cagliani 2015). Over the last 5 years, about 20 papers have been published on honey characterisation, a few of which dealt specifically with chemical markers in mānuka honey. Donarski et al. determined for the first time, without chromatographic separation or derivatisation, the total MGO by quantifying the two hydrates of MGO formed in aqueous solution (Donarski et al. 2010). Although the amount of MGO does not uniquely designate a sample as mānuka, the method is of value, as MGO content correlates with a honey's antimicrobial strength measured by the 'Unique Mānuka Factor' (UMF) (Allen et al. 1991). Diffusion ordered spectroscopy (DOSY) NMR for the virtual separation of key components of mānuka honey has been shown to be of potential for the discriminatory analysis, although most of the compounds giving marker signals were not identified (Le Gresley et al. 2012). Improved resolution in diffusion NMR is possible through the application of the homonuclear decoupled 'Pureshift DOSY' variant (Nilsson and Morris 2007); its application to honey authenticity would be interesting.

The combination of ^{1}H NMR and chemometrics has been shown to discriminate mānuka honey from other floral honey types from Oceania (Spiteri et al. 2017). The known mānuka markers, MGO DHA and leptosperin, can be determined simultaneously. In addition, it was possible to detect an NMR resonance marker (the originating compound is as-yet unidentified for Kamahi (*Weinmannia racemosa*), another native New Zealand plant, which flowers at the same time as mānuka and markers from eucalyptus honeys). Although New Zealand cannot import honey for blending, this marker would prevent New Zealand honey being mixed with Australian honey and fraudulently being labelled as produced in New Zealand.

Fluorescence

Ruoff et al. made a preliminary study of the botanical origin of honey by front-face fluorescence spectroscopy in 2005 (Ruoff et al. 2005). The front-face mode was chosen to avoid distortion of the emission spectra and reduction of intensity from inner-filter effects with samples with high absorbancy. They followed up with a more detailed study, using predominantly Swiss honeys, and showed good discrimination between honey types (Ruoff et al. 2006). Karoui et al. made a similar study to classify Swiss honeys (Karoui et al. 2007).

The first report of the potential of fluorescence to determine the botanical origin with specific reference to mānuka and kānuka is a patent using a two-dimensional excitation-emission matrix (Aikenhead et al. 2010) which cited only Karoui et al.

Two fluorescent pteridine derivatives were found to discriminate between mānuka and the pollen identical kānuka honey using HPLC or more simply by TLC with a fluorescence visualizer (Beitlich et al. 2016).

Using the Aikenhead et al. concept of excitation/emission (ex-em) marker wavelengths, two sets were identified for mānuka and for kānuka honeys: for mānuka honey, ex-em was at 270–365 nm (MM1) and at 330–470 nm (MM2), and for kānuka honey, at 275–305 nm (KM1) and at 445–525 nm (KM2) (Bong et al. 2016). The origin of the MM1 marker has since been shown to be leptosperin (Bong et al. 2017) also known as leptosin (Kato et al. 2012). The origin of MM2 has been shown to be lepteridine (Lin et al. 2017) and for which a synthetic route is available (Daniels et al. 2016).

Field Tests for Leptosperin

Immuno-chromatography can be used outside a laboratory environment with little specialised training and produces results within minutes. Kato et al. (2016) showed that it was possible to produce such a kit to test the authenticity of mānuka honey. Anti-leptosperin antibody was raised and conjugated to 40 nm colloidal gold and vacuum dried prior to storage in glass vials. Diluted honey was added to the specific antibody conjugated to colloidal gold and applied in a lateral flow assay with leptosperin–bovine serum albumin as the test line and goat anti-mouse IgG as the control line. The ELISA on which the immuno-chromatographic kit was based yielded results that correlated well with prior HPLC studies (Kato et al. 2014a).

Discussion

To determine the purity of a given sample labelled a mānuka honey using its content of specific chemical markers, it is necessary to know their normal concentration range in authentic pure samples, both within seasons and from year to year. Authentic samples from the apiaries, in sufficient numbers and chemically characterised for the parameters laid down in the EU Directive (EU Council 2002), and by the methods we suggest herein, are necessary.

The use of DNA from pollen for food control analysis is less likely to be applied due to the costs, time delay to acquire data and in addition its inability to provide quantitative measurement of absolute purity of mānuka or other honey samples.

Of the various chemical compounds found in samples of mānuka honey, two namely leptosperin and lepteridine are unique and readily determinable. However, no normal range data is currently available for leptosperin; however, for lepteridine, Daniels et al. (2016) have shown that its content varies greatly by location (5.23–39.29 μg/g) and in nectar, for three glasshouse grown, *L. scoparium* varieties (43.70–79 μg/g). Thus, to interpret the lepteridine content in terms of the purity of a sample will require knowledge of the effects of the distribution of *L. scoparium* varieties in the geographical area of origin of the honey in addition to the normal range for each species. If the same data becomes available for leptosperin, then NMR would be a good choice of methodology in that it can in addition to the purity provide in a single run the concentration of MGO which provides a measure of the UMF for the sample (Donarski et al. 2010).

Fluorescence spectrometry is a fast and cheap technique and has the advantage of being able to provide the concentration of both not only the unique chemical marker concentrations but also the presence of two markers for kānuka although confirming a less than 100% pure mānuka would indicate a New Zealand-produced product.

If an immuno-chromatographic test became commercially available, it would be useful for on-site control of honey samples by beekeepers, food inspectors, honey exporters and importers alike.

Conclusions

The following suggestions, based on gaps in data available in the public domain, are made to provide rapid and cost-effective authentication of samples stated to be mānuka honey.

(a) International validation of an NMR method for the determination of leptosperin and total MGO in samples if possible incorporate determination of the second marker lepteridine.
(b) Establish the range of expected values for the markers to be used to establish the percent of purity of samples of mānuka honey, bearing in mind the distribution of the various varieties of *L. scoparium* in the various honey-producing areas in New Zealand.
(c) Encourage the development of a validated point of use immuno-chemical test kit for leptosperin.
(d) Further studies in the application of front-surface fluorescence are indication as this method allows the simultaneous measurement of chemical makers for kānuka honey in addition to those for mānuka honey.

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Conflict of Interest D. Thorburn Burns declares that he has no conflict of interest. A. Dillon declares that she has no personal interest. J. Warren declares that he has no personal interest. M. J. Walker declares that he has no personal interest.

Ethical Consent This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

References

Adams CJ, Boult CH, Deadman BJ, Farr FM, Grainger MNC, Manley-Harris M, Snow MJ (2008) Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey. Carbohydr Res 343(4):651–659. https://doi.org/10.1016/j.carres.2007.12.011

Adams CJ, Manley-Harris M, Molan PC (2009) The origin of methylglyoxal in New Zealand Manuka (Leptospermum scoparium) honey. Carbohydr Res 344(8):1050–1053. https://doi.org/10.1016/j.carres.2009.03.020

Aikenhead C, Rosedale D, Schlothauer RC, Stephens JMD (2010) A method and apparatus that utilises fluorescence to determine plant or botanical origin characteristics of honey. PCT/NZ 2011/0002489, filed 29.11.2010

Aitken HRM, Johannes LKM, Brimble MA (2013) Synthesis of leptosin, a glycoside isolated from mānuka honey. Tetrahedron Lett 54(50):6916–6919. https://doi.org/10.1016/j.tetlet.2013.10.042

Allen KL, Molan PC, Reis GM (1991) A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 43(12):817–822. https://doi.org/10.1111/j.2042-7158.1991.tb03186.x

Anklam E (1997) A review of analytical methods to determine the geographical and the botanical origin of honey. Food Chem 63:540–562

Aries E, Burton J, Carrasco L, De Rudder O, Masquet A (2016) Scientific support to the implementation of a coordinated control plan with a view to establishing the prevalence of fraudulent practices in the marketing of honey: results of honey authenticity testing by liquid chromatography-isotope ratio mass spectrometry, JCR Technical Report No. SANTE/2015/E3/JCR/S12.706828

Atrott J, Haberlau S, Henle T (2012) Studies on the formation of methylglyoxal from dihydroxyacetone in Manuka (Leptospermum scoparium) honey. Carbohydr Res 361:7–11. https://doi.org/10.1016/j.carres.2012.07.025

Beitlich N, Lűbken T, Kaiser M, Ispiryan L, Speer K (2016) Fluorescent pteridine derivatives as new markers for the characterisation of genuine monofloral New Zealand manuka (Leptospermum scoparium) honey. J Agric Food Chem 64(46):8886–8891. https://doi.org/10.1021/acs.jafc.6b03984

Bong J, Loomes KM, Schlothauer RC, Stephens JM (2016) Fluorescence markers in some New Zealand honeys. Food Chem 192:1006–1014. https://doi.org/10.1016/j.foodchem.2015.07.118

Bong J, Prijic G, Braggins TJ, Schlothauer RC, Stephens JM, Loomes KM (2017) Leptosperin is a distinct and detectable fluorophore in Leptospermum honeys. Food Chem 214:102–107. https://doi.org/10.1016/j.foodchem.2016.07.018

Bruni GA, Caridi L, Scaccabarozzi D, De Mattia F, Casiraghi M, Labra M (2015) A DNA barcoding approach to identify plant species in multiflower honey. Food Chem 170:308–315. https://doi.org/10.1016/j.foodchem.2014.08.060

Codex Alimentarius (1981) International Food Standards, Standard For Honey CODEX STAN 12-1981, last revised 2001

Consonni R, Cagliani LR (2015) Recent developments in honey characterisation. RSC Adv 5(73):59696–59714. https://doi.org/10.1039/C5RA05828G

Daniele G, Maitre D, Casabianca H (2012) Identification, quantification and carbon stable isotopes determinations of organic acids in monofloral honeys. A powerful tool for botanical and authenticity control. Rapid Commun Mas Spectrom 26(17):1993–1998. https://doi.org/10.1002/rcm.6310

Daniels BJ, Prijic G, Meidinger S, Loomes KM, Stephens JM, Schlothauer RC, Furkert DP, Brimble MA (2016) Isolation, structural elucidation, and synthesis of lepteridine from mānuka (Leptospermum scoparium) honey. J Agric Food Chem 64(24):5079–5084. https://doi.org/10.1021/acs.jafc.6b01596

Donarski JA, Roberts DPT, Charlton AJ (2010) Quantitative NMR spectroscopy for the rapid measurement of methylglyoxal in manuka honey. Anal Methods 2(10):1479–1483. https://doi.org/10.1039/c0ay00125b

E.U. Council (2002) Council Directive 2001/110/EC of 20 December 2001 relating to honey. Off J Eur Communities 10:47–52

Elflein L, Raezke K-P (2008) Improved detection of honey adulteration by measuring differences between δ13C/δ12C stable carbon isotope ratios of protein and sugar compounds with a combination of elemental analyzer—isotope ratio mass spectrometry and liquid chromatography—isotope ratio mass spectrometry (δ13C-EA/LC-IRMS). Apidologie 39:574–587

European Commission (2015) Commission Recommendation of 12.3.2015. On a coordinated control plan with a view to establishing the prevalence of fraudulent practices in the marketing of certain foods. Annex 1, honey authenticity. EU Brussels

Fairchild GF, Nichols JP, Capps O Jr (2003) Observations on economic adulteration of high-value food products: the honey case. J Food Distrib Res 34:38–45

Fearnley L, Greenwood DR, Schmitz M, Stephens JM, Schlothauer RC, Loomes KM (2012) Compositional analysis of manuka honeys by high-resolution mass spectrometry: identification of a Manuka-enriched archetypal molecule. Food Chem 13:948–953

Guertler P, Eicheldinger A, Muschler P, Goerlich O, Busch U (2014) Automated DNA extraction from pollen in honey. Food Chem 149:302–306. https://doi.org/10.1016/j.foodchem.2013.10.129

Hawkins J, de Vere N, Griffith A, Ford CR, Allainguillaume J, Hegarty MJ, Baillie L, Adams-Groom B (2015) Using DNA metabarcoding to identify the floral composition of honey: a new tool for investigating honey bee foraging preferences. PloS One 10:e0134735

Jain SA, Jesus FTD, Marchioro GM, Araújo EDD (2013) Extraction of DNA from honey and its amplification by PCR for botanical identification. Food Sci Technol (Campinas) 33(4):753–756. https://doi.org/10.1590/S0101-20612013000400022

Jandrić Z, Frew RD, Fernandez-Cedi LN, Cannavan A (2017) An investigative study on discrimination of honey of various floral and geographical origins using UPLC-QToF MS and multivariate data

analysis. Food Control 72:189–197. https://doi.org/10.1016/j.foodcont.2015.10.010

Karoui R, Dufour E, Bosset J-O, Baerdemaeker (2007) The use of front face fluorescence spectroscopy to classify the origin of honey samples produced in Switzerland. Food Chem 101(1):314–323. https://doi.org/10.1016/j.foodchem.2006.01.039

Kato Y, Umeda N, Maeda A, Matsumoto D, Kitamoto N (2012) Identification of a novel glycoside, Leptosin, as a chemical marker of manuka honey. J Agric Food Chem 60(13):3418–3423. https://doi.org/10.1021/jf300068w

Kato Y, Araki Y, Jur M, Fujinaka R, Ishisala A, Kitamoto N, Hitta Y, Niwa T, Takimoto Y (2014a) Immunochemical authentication of manuka honey using a monoclonal antibody specific to a glycoside of methyl syringate. J Agric Food Chem 62(44):10672–10678. https://doi.org/10.1021/jf503464a

Kato Y, Fujinaka R, Ishisaka NY, Kitamoto N, Takimoto (2014b) Plausible authentication of manuka honey and related products by measuring leptosperin with methyl syringate. J Agric Food Chem 62(27):6400–6407. https://doi.org/10.1021/jf501475h

Kato Y, Araki Y, Juri M, Ishisaka A, Nitta Y, Niwa T, Kitamoto N, Takimoto Y (2016) Competitive immunochromatographic assay for leptosperin as a plausible authentication marker of manuka honey. Food Chem 194:362–365. https://doi.org/10.1016/j.foodchem.2015.08.040

Kolayli S, Boukraâ L, Şahin H, Abdellah F (2012) Sugars in honey. In: Preedy VR (ed) Dietary sugars: chemistry, analysis, function and effects. Royal Society of Chemistry, Cambridge, pp 3–15. https://doi.org/10.1039/9781849734929-00003

Lalhmangaihi R, Ghatak S, Laha R, Gurusubramanian G, Kumar NS (2014) Protocol for optimal quality and quantity pollen DNA isolation from honey samples. J Biomol Techn: JBT 25(4):92–95. https://doi.org/10.7171/jbt.14-2504-001

Le Gresley A, Kenny J, Cassar C, Kelly A, Sinclair A, Fielder MD (2012) The application of high resolution diffusion NMR to the analysis of manuka honey. Food Chem 135(4):2879–2886. https://doi.org/10.1016/j.foodchem.2012.07.072

Leake G (2017) Bee careful: that honey may be fake. The Sunday Times, 5th. February, London, p 5

Lin B, Loomes KM, Prijic G, Schlothauer R, Stephens JM (2017) Lepteridine as a unique fluorescent marker for the authentication of manuka honey. Food Chem 225:175–180. https://doi.org/10.1016/j.foodchem.2016.12.099

Manyi-Loh CE, Ndip RN, Clarke AM (2011) Volatile compounds in honey: a review of their aroma, botanical origin determination and potential biomedical activities. Int J Mol Sci 12(12):9514–9532. https://doi.org/10.3390/ijms12129514

Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Mol Nutr Food Res 52(4):483–489. https://doi.org/10.1002/mnfr.200700282

Moore JC, Spink J, Lipp M (2012) Development and application of a database for food ingredient fraud and economically motivated adulteration from 1980-2010. J Food Sci 77:R119–R126

MPI (2013) Options for defining monofloral manuka honey. MPI Discussion Paper No:2013/38. MPI New Zealand Government

MPI (2014) Science and characterising mānuka honey. Current and future science to support a definition. MPI Technical Paper No:2014/23. MPI, New Zealand Government

MPI (2017a) Criteria for identifying mānuka honey. MPI Technical Paper No: 2017/28. MPI, New Zealand Government

MPI (2017b) Determination of four chemical characterisation compounds in honey by liquid chromatography tandem mass spectrometry (LC-MS/MS). MPI Technical Paper No: 2017/30. MPI, New Zealand Government

MPI (2017c) Multiplex qPCR for detection of *Leptospermum scoparium* DNA from pollen in Honey. MPI Technical Paper No 2017/31. MPI New Zealand Government

MPI (2017d) MPI, New Zealand Government, Mānuka honey. http://www.mpi.govt.nz/growing-and-harvesting/honey-and-bees/manuka-honey/. Accessed 22 Dec 2017

Nilsson M, Morris GA (2007) Pure shift proton DOSY: diffusion-ordered 1H spectra without multiplet structure. Chem Commun (9):933–935. https://doi.org/10.1039/b617761a

Prosser SW, Hebert PD (2017) Rapid identification of the botanical and entomological sources of honey using DNA metabarcoding. Food Chem 214:183–191. https://doi.org/10.1016/j.foodchem.2016.07.077

Rogers KM, Sim M, Stewart S, Phillips A, Cooper J, Douance C, Pyne R, Rogers P (2014) Investigating C-4 sugar contamination of Manuka honey and other New Zealand honey varieties using carbon isotopes. J Agric Food Chem 62(12):2605–2614. https://doi.org/10.1021/jf404766f

Ruoff K, Karoui R, Dufour E, Luginbűhl W, Bosset J-L, Bogdanov S, Amadò R (2005) Authentication of the botanical origin of honey by front-face spectroscopy. A preliminary study. J Agric Food Chem 54:1343–1347

Ruoff K, Luhinbűhl W, Kűnzli R, Bogdanov S, Bosset JL, von der Ohe K, von der Ohe W, Amadò R (2006) Authentication of the botanical and geographical origin of honey by front-face fluorescence spectroscopy. J Agric Food Chem 54(18):6858–6866. https://doi.org/10.1021/jf060697t

Soares S, Amaral JS, Oliveira MBP, Mafra I (2015) Improving DNA isolation from honey for the botanical origin identification. Food Control 48:130–136. https://doi.org/10.1016/j.foodcont.2014.02.035

Spiteri M, Rogers KM, Jamin E, Thomas F, Guyader S, Lees M, Rutledge DN (2017) Combination of 1H NMR and chemometrics to discriminate manuka honey from other floral honey types from Oceania. Food Chem 217:766–777. https://doi.org/10.1016/j.foodchem.2016.09.027

Torricelli et al (2016) In-house validation of a DNA extraction protocol from honey and bee pollen and analysis in fast rea-time PCR of commercial honey samples using a knowledge based approach. Food Anal Methods 9(12):3439–3450. https://doi.org/10.1007/s12161-016-0539-x

Wallace A, Eady S, Miles M, Martin H, McLachlan A, Rodier M, Willis J, Scott R, Sutherland J (2010) Demonstrating the safety of manuka honey UMF® 20+ in a human clinical trial with healthy individuals. Brit. J Nutr 103:1013–1028

Weigel KU, Opitz T, Henle T (2004) Studies on the occurrence and formation of 1,2-dicarbonyls in honey. Eur Food Res Technol 218(2):147–515. https://doi.org/10.1007/s00217-003-0814-0